

***Intsia palembanica* wood extracts and its isolated compounds as *Propionibacterium acnes* lipase inhibitor**

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Abstract Potential compounds from *Intsia palembanica* methanol extracts were isolated. Three isolated compounds as well as 7 reported compounds from Merbau were analyzed for their ability to inhibit the lipase activity of *Propionibacterium acnes*. Lipase was isolated from *P. acnes* and used as enzyme for activity analysis. The lipase activity test was performed using the 2,3-dimercapto-1-propanol tributyrate (BALB) method. The results showed that methanol extract and water fraction were active to inhibit lipase activity but *n*-hexane fraction and EtOAc fraction did not reach 50 % inhibition until 500 µg/ml. EtOAc fraction consisted of flavonoid, naringenin, robinetin, and (+)-epirobidanol accelerated the lipase activity. The other 7 compounds showed inhibitory activity of lipase in a concentration-dependent manner. In conclusion, fustin is the most active compound to inhibit the *P. acnes* lipase activity.

Keywords *Intsia palembanica* · *Propionibacterium acnes* lipase inhibitor · Flavonoid

Introduction

Propionibacterium acnes is a gram-positive anaerobic bacteria which is implicated in the inflammatory phase of acne. It secretes several proinflammatory products such as lipases, proteases hyaluronidases, and chemotactic factors related to the development of inflammation in acne [1]. *Propionibacterium acnes* lipases produce free fatty acids on sebaceous triglycerides that finally results to severe inflammation [2].

Previous research reported that one of 40 Indonesia plant materials, *Intsia palembanica* (Merbau), had a strong *P. acnes* lipase inhibition activity. Despite having no antibacterial activity against *P. acnes*, the Merbau methanolic extract gave a very strong lipase inhibition activity as well as a very high anti-oxidant activity [3]. Other natural substances such as Kampo formulation, coptidis rhizome and its alkaloid, catechin, and kaempferol have been reported to inhibit *P. acnes* lipase activity [4, 5]. Unfortunately, the publication related to *P. acnes* lipase inhibitory activity from natural product is still limited, so that it is interesting to look for the active compounds from Merbau for *P. acnes* lipase inhibition.

Ten flavonoid compounds: (–)-robidanol, (+)-epirobidanol, 4'-dehydroxyrobidanol, fustin, ampelopsin, naringenin, robinetin, myricetin, quercetin and 3,7,3',5'-tetrahydroxyflavone were isolated from Merbau methanolic extracts [6, 7]. The structures of the 10 compounds are shown in Fig. 1. On this paper the activity of methanol extract, *n*-hexane fraction, EtOAc fraction, water fraction, and 10 isolated flavonoids against *P. acnes* lipase will be discussed.

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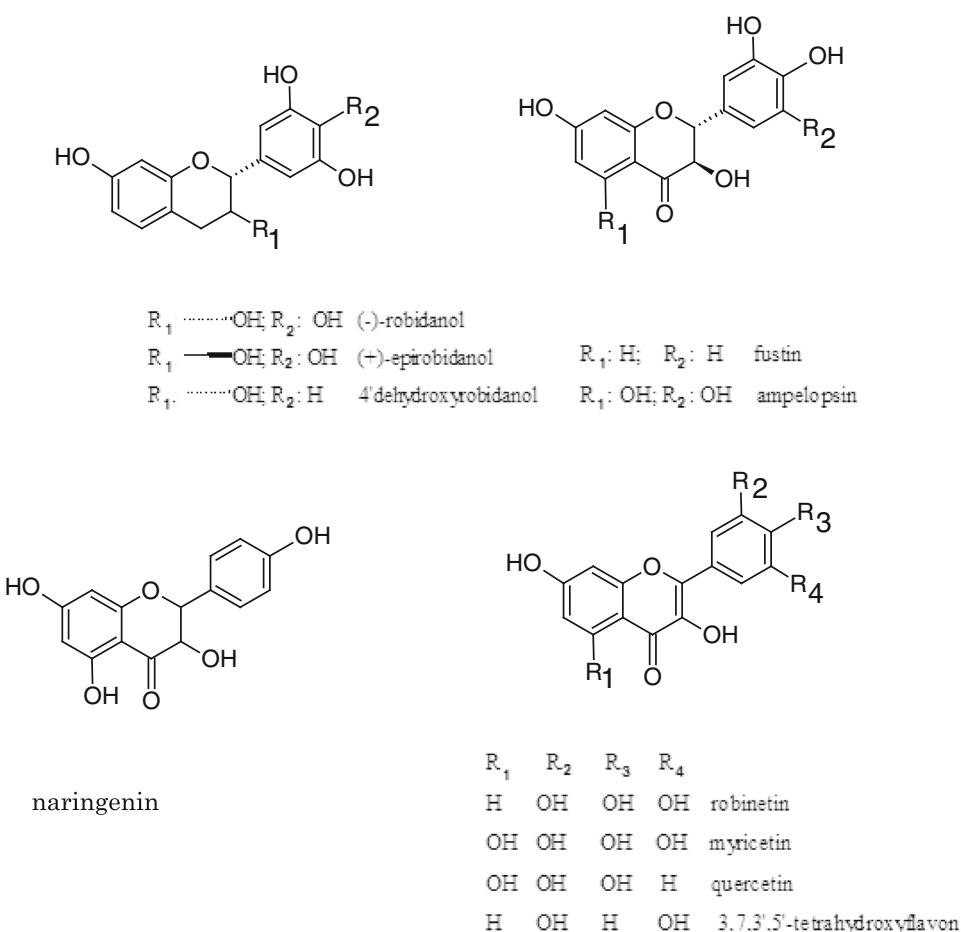
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Fig. 1 Structures of the flavonoids from *I. palembanica*



Materials and methods

Plant material Merbau was collected from East Kalimantan, Indonesia. The identification and voucher specimen was deposited at the Wood Anatomy Laboratory, Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia No. FHT.LA. 12.9p.

Chemicals Quercetin (Sigma Aldrich), naringenin (Sigma Aldrich), myricetin (Extrasyntheses, Genay, France), fustin, ampelopsin, and robinetin compounds were collected and isolated previously.

Extraction and isolation

The extraction, fractionation and isolation methods were performed as previous reported described [7]. The methanol extract, *n*-hexane fraction, EtOAc fraction, and water fraction were obtained. Finally, three compounds: (−)-robidanol (purity of 96 % based on HPLC), (+)-epirobidanol (purity of 95 % based on HPLC), and 4'-dehydroxyrobidanol (purity of 91 % based on HPLC) were isolated and determined from EtOAc fractions.

Lipase inhibitory activity assay

The lipase used on this research was isolated from *P. acnes* as described in our previous report. Lipase inhibitory activity assay was also conducted using the 2,3-dimercapto-1-propanol tributyrate (BALB) method previously described [3]. Tetracycline was used as the positive control based on its activity to inhibit the lipase production of *P. acnes* [8]. The samples used were methanol extracts, *n*-hexane fraction, EtOAc fraction, water fraction, and 10 flavonoids from Merbau. The concentration used for the samples ranged from 2.0 to 500 µg/ml. The activity of each sample is reported as % inhibition. We selected 5 different concentrations and reported in this paper.

Results and discussion

Methanol extract of *I. palembanica* had a very strong inhibitory activity against *P. acnes* lipase (IC_{50} : 4.1 µg/ml) [3]. At the concentration of 62.5 µg/ml it inhibited about 70 % *P. acnes* lipase activity (Fig. 2). The fractionation of this extract into *n*-hexane, EtOAc and water gave different

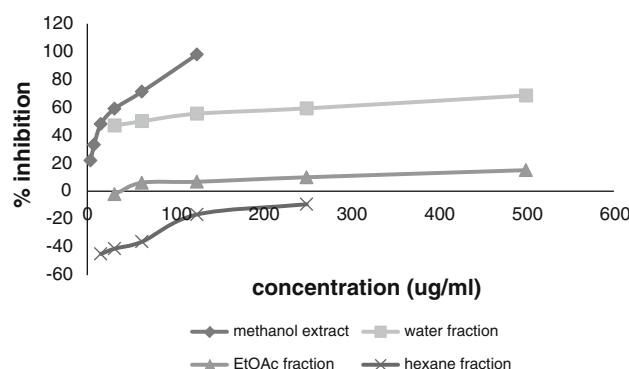


Fig. 2 Inhibition of lipase activities of methanol extract, *n*-hexane fraction, EtOAc fraction, and water fraction at different concentrations

results. Non-polar fraction (*n*-hexane fraction) accelerated the lipase activity on a concentration-dependent manner; lower concentrations gave accelerated activities. The semi-polar fraction (EtOAc fraction), however only inhibited 15 % *P. acnes* lipase activity at the high concentration of 500 µg/ml. The active fraction found in the polar fraction (water fraction) could inhibit 50 % lipase activity at the concentration of 62.5 µg/ml (IC₅₀: 48.64 µg/ml, Table 1).

Activity of methanol extract and other fractions are different. The differences could be explained by HPLC chromatogram (Fig. 3). The *n*-hexane fraction consisted of non-polar compounds which is not active to inhibit the lipase activity. A chromatogram of this fraction has peaks after 40 min. In a ODS column chromatography, the lower polarity of the compound the later the elution time. Therefore these peaks appearing at later retention time would be a non polar compound. The yield of *n*-hexane fraction is 0.39 % from methanol extract and only gives small effect to the methanol extract. The EtOAc fraction with yield about 40 % from methanol extract has peaks between retention times of 17–30 min. This peaks consisted of some flavonoids such as epirobidanol (retention time of 17.2 min), ampelopsin (retention time of 20.1 min), robidanol (retention time of 21.1 min), 4'-

dehydroxyrobidanol (retention time of 22.6 min), myristin (retention time of 29.2 min), etc. Based on the activity of EtOAc fraction, it could be assumed that most of flavonoid in EtOAc fraction could be not active and others could have activities. So, it is interesting to study about the activity of some flavonoid in *I. palembanica* against lipase. The water fraction which is main component in methanol extract (yield 58 % from methanol extract) is the responsible fractions for the activity of *I. palembanica* methanol extract. Based on the peak shape of water fraction, the water fraction could be consists of tannins. Tannins have been reported as lipase inhibitory active compounds [9].

The lipase inhibitory activity of extract, fraction as well as the flavonoid compounds are compared with tetracycline. Based on the ability of tetracycline as an antimicrobial and its inhibitory activity against *P. acnes* lipase, tetracycline is used as positive controls [2]. Event tetracycline reported has activity to inhibit lipase, but this research results showed that tetracycline is not good enough to inhibit lipase from *P. acnes*.

The 10 flavonoid isolated compounds could be divided into 4 subgroups, namely: flavan-3-ols, flavanone, flavon, and flavonol. (–)-robidanol, (+)-epirobidanol, and 4'-dehydroxyrobidanol belong to the flavan-3-ols group, whereas fustin and ampelopsin belongs to the flavanone group. Only naringenin belongs to the flavon group while robinetin, myricetin, quercetin and 3,7,3',5'-tetrahydroxyflavone are classified under the flavonol group. These compounds have different activities against *P. acnes* lipase (Fig. 4). Three compounds: (+)-epirobidanol, robinetin and naringenin, accelerated the lipase activity, while another 7 compounds inhibited the lipase activity in a concentration-dependent manner. The IC₅₀ lipase inhibition values of the compounds are listed in Table 1.

The flavan-3-ol group, (–)-robidanol and 4'-dehydroxyrobidanol have lipase inhibitory activities while the (+)-epirobidanol accelerated the lipase activity. The stereochemistry becomes important in the inhibition activity. (+)-catechin which belongs to the flavan-3-ol group but has the same stereochemistry with (+)-epirobidanol could

Table 1 IC₅₀ values of fractions and compounds derived from *Intsia palembanica* extracts

Sample name	IC ₅₀ (µg/ml)	Compound name	IC ₅₀	
			(µg/ml)	(µM)
Methanol extract	4.10 ± 0.21	(–)-robidanol	29.06 ± 5.84	100.2 ± 20.1
<i>n</i> -hexane fraction	>500	4'-dehydroxyrobidanol	10.97 ± 0.79	40.0 ± 2.8
EtOAc fraction	>500	Ampelopsin	10.97 ± 2.11	36.1 ± 6.9
Water fraction	48.64 ± 5.82	Fustin	3.95 ± 0.24	13.7 ± 0.8
		Quercetin	127.26 ± 4.11	421.4 ± 13.6
		myricetin	107.40 ± 3.52	375.5 ± 12.3
		3,7,3',5'-tetrahydroxyflavone	239.11 ± 3.53	885.6 ± 12.9
		Tetracycline	471.34 ± 13.42	1,060 ± 53.4

Fig. 3 HPLC profiles of methanol extract (a), *n*-hexane fraction (b), EtOAc fraction (c) and water fraction (d)

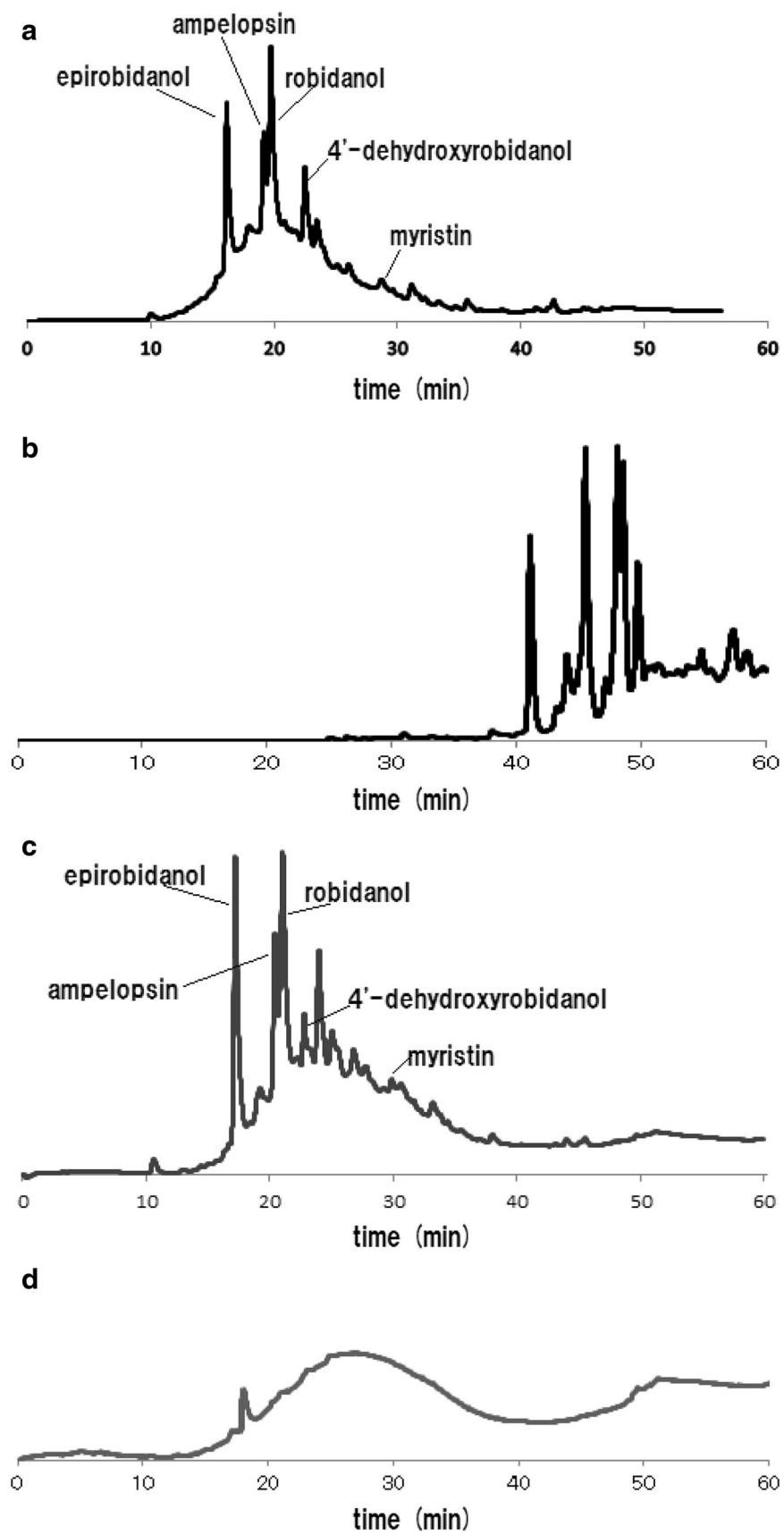
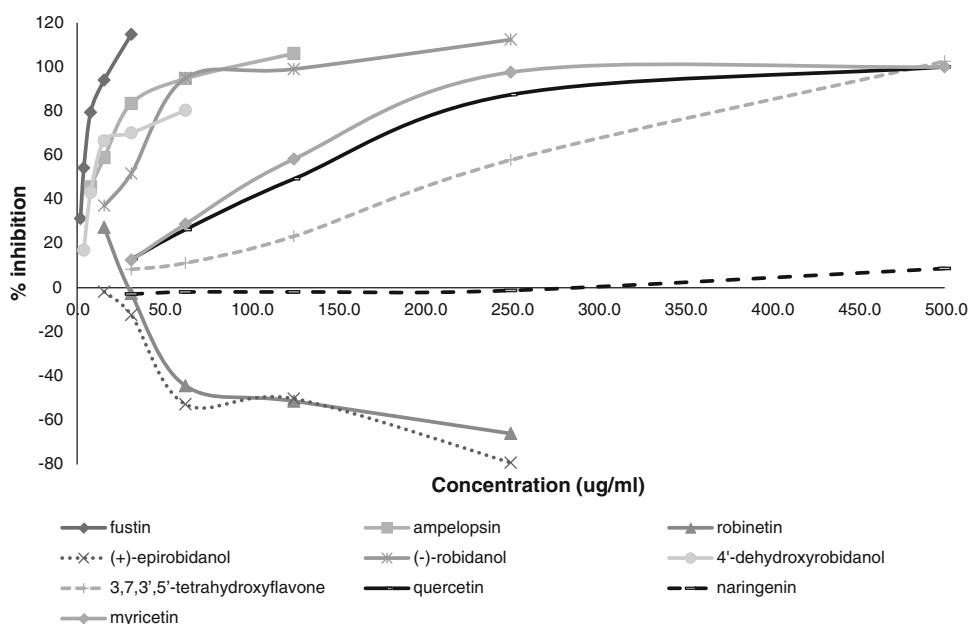


Fig. 4 Lipase inhibitory activities of flavonoid compounds from *I. palembanica*



not inhibit the lipase activity on the concentration of 862 μM [10] and it was reported that the IC_{50} value was 39 mM [5]. In addition 4'-dehydroxyrobidanol is more active compared to (−)-robidanol based on the IC_{50} value. This indicates that the hydroxyl group in Ring-B has also contributes to the lipase inhibition activity.

Based on IC_{50} value, fustin (13.7 μM) is the most active compound among the 10 compounds. The fustin concentration needed to inhibit 50 % lipase activity is 3 times lower compared to ampelopsin (IC_{50} : 36.1 μM) which has more hydroxyl groups (in C-5 and 5'). The IC_{50} value of ampelopsin is not significantly different with 4'-dehydroxyrobidanol (IC_{50} : 40.0 μM).

Naringenin which belongs to flavone group has no bacterial lipase inhibition activity. At low concentrations, naringenin accelerated the activity of *P. acnes* lipase and at high concentrations it showed very weak lipase inhibition activity (Fig. 4).

In the flavonol group, robinetin accelerated the lipase activity while the 3 other flavonol compounds inhibited lipase activity at different concentrations. Myricetin and quercetin had almost the same activity in almost all concentrations used (Fig. 4). The IC_{50} values of myricetin and quercetin are significantly different ($p < 0.05$); myricetin is more active compared to quercetin. Quercetin is reported to inhibit lipase from bacteria especially against *Candida rugosa* lipase but the IC_{50} value was higher than 80 mM [11]. Unlike the two compounds, 3,7,3',5'-tetrahydroxyflavone inhibited the *P. acnes* lipase activity at a high concentration. The existence of a hydroxyl group in C-5 of flavonol (myricetin, quercetin) becomes important; without it, the reverse will apply as what happened in robinetin and 3,7,3',5'-tetrahydroxyflavone. The IC_{50} value of 23 mM for

kaempferol had been reported against *P. acnes* lipase [5], but this value is higher than myricetin, quercetin and 3,7,3',5'-tetrahydroxyflavone.

Out of the 10 flavonoids, fustin, ampelopsin and 4'-dehydroxyrobidanol are the most active lipase inhibitory compounds. Their IC_{50} values are significantly different with tetracycline as the positive control. In addition, these compounds are more active compared to other compounds reported against *P. acnes* lipase such as chebulagic acid (IC_{50} : 60.0 μM), tannic acid (IC_{50} : 129 μM) and ellagic acid (IC_{50} : 298 μM) [12].

Conclusion

The water fraction of methanol extract is the most active fraction from *I. palembanica* methanol extract with IC_{50} of 48.64 $\mu\text{g/ml}$. The other fraction, *n*-hexane fraction and EtOAc fraction are not active. Out of the 10 compounds from *I. palembanica* from EtOAc fraction, (+)-epirobidanol, robinetin, and naringenin are lipase activity accelerators. The inhibitor of the lipase produced by *P. acnes* are fustin (IC_{50} of 13.7 μM), ampelopsin (IC_{50} of 36.1 μM), and 4'-dehydroxyrobidanol (IC_{50} of 40.0 μM). These three compounds are promising anti-acne agents through the mechanism of lipase inhibition.

References

1. Heymann WR (2006) Dialogue in dermatology: toll-like receptors in acne vulgaris. *J Am Acad Dermatol* 55:691–692

2. Higashi S (2003) Lipase inhibitors for the treatment of acne. *J Mol Catal B Enzym* 22:377–384
3. Batubara I, Mitsunaga T, Ohashi H (2009) Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. *J Wood Sci* 55:230–235
4. Higaki S, Nakamura M, Morohashi M, Hasegawa Y, Yamagishi T (1996) Anti-lipase activity of Kampo formulations, coptidis rhizome and its alkaloids against *Propionibacterium acnes*. *J Dermatol* 23(5):310–314
5. Falcocchio S, Ruiz C, Pastor FIJ, Saso L, Diaz P (2006) *Propionibacterium acnes* GehA lipase, an enzyme involved in acne development, can be successfully inhibited by defined natural substances. *J Mol Catal B Enzym* 40:132–137
6. Imamura H, Nomura K, Hibino Y, Ohashi H (1974) A new type of flavonol im the “shake” of Merbau wood. *Mokuzai Gakkaishi* 20(3):143–144
7. Batubara I, Darusman LK, Mitsunaga T, Aoki H, Rahminiati M, Djauhari E, Yamauchi K (2011) Flavonoid from *Intsia palembanca* as skin whitening agent. *J Biol Sci* 11(8):475–480
8. Websters GF, McGinley KJ, Leyden JJ (1981) Inhibition of lipase production in *Propionibacterium acnes* by sub-minimal-inhibitory concentrations of tetracycline and erythromycin. *Br J Dermatol* 104(4):453–457
9. Muddathir AM, Yamauchi K, Mitsunaga T (2013) Anti-acne activity of tannin-related compounds isolated from *Terminalia laxiflora*. *J Wood Sci* 59:426–431
10. Batubara I, Mitsunaga T, Ohashi H (2010) Brazilin from *Caesalpinia sappan* wood as antiacne agent. *J Wood Sci* 56:77–81
11. Gatto MT, Falcocchio S, Grippa E, Mazzanti G, Battinelli L, Nicolosi G, Lambusta D, Saso L (2002) Antimicrobial and anti-lipase activity of quercentin and its C2–C16 3-O-acyl-ester. *Bioorg Med Chem* 10:269–272
12. Patil V, Bandivadekar A, Debjani D (2012) Inhibition of *Propionibacterium acnes* lipase by extracts Indian medicinal plants. *Int J Cosmet Sci* 34:234–239